

APPENDIX I
FORMULATION OF PRODUCTS

A. Formulation of the Emulsion Base

1. Protocol

- 1 Add cold water and commence agitation
- 2 Heat water to 82 C
- 3 Add EDTA, Na Benzoate, Ceteareth-25
- 4 Add Stearyl/Cetyl Alcohol and Phenoxyethanol
- 5 Hold for 45 Mins at 80 C
- 6 Cool to 60 C whilst milling at 4000rpm using Turrax T50
- 7 At 60 C, stop milling and continue cooling to 50 C
- 8 Hold at 50 C for 1 hour
- 9 Check pH and allow to cool to 30 C
- 10 Pack off product

2. Ingredients

<u>Description</u>	<u>Wt %</u>
DI Water	82.6833
Cetyl Alcohol	6.2500
Stearyl Alcohol	6.2500
Ceteareth 25	4.1667
Phenoxyethanol	0.3000
Sodium Benzoate	0.2500
Tetra Sodium EDTA	0.1000

B. Formulation of Final Products

1. Protocol

- 1 Add water
- 2 Add chelant
- 3 Add ammonium hydroxide
- 4 Add peracetic acid
- 5 pH adjust with acetic acid to required pH
- 6 Add solution to emulsion base slowly and mix thoroughly
- 7 Qs with DI water

8 Add hydrogen peroxide

2. Ingredients

a. Product 1

<u>Description</u>	<u>Wt %</u>
Emulsion Base	35.0000
Demineralised water	35.0000
Peracetic Acid (35% active)	3.1400
Ammonium Hydroxide (30% active)	4.0000
Hydrogen Peroxide (35% active)	11.5500
EDDS chelating agent	6.3000
pH adjust with Acetic Acid to pH 8	qs
qs to 100% with DI water	qs

b. Product 2

<u>Description</u>	<u>Wt %</u>
Emulsion Base	35.0000
Demineralised water	35.0000
Peracetic Acid (35% active)	3.1400
Ammonium Hydroxide (30% active)	4.0000
Hydrogen Peroxide (35% active)	11.5500
EDTA chelating agent	0.1000
pH adjust with Acetic Acid to pH 10	qs
qs to 100% with DI water	qs

c. Product 3

<u>Description</u>	<u>Wt %</u>
Emulsion Base	35.0000
Demineralised water	40.0000
Peracetic Acid (35% active)	3.1400
Ammonium Hydroxide (30% active)	4.0000
Hydrogen Peroxide (35% active)	11.5500
EDTA chelating agent	0.1000
pH adjust with Ammonia to pH 10	qs
qs to 100% with DI water	qs

d. Product 4

i. Part A

<u>Description</u>	<u>Wt %</u>
Emulsion Base	35.0000
Demineralised water	38.6500
DTPA chelating agent	0.2500
HEDP chelating agent	0.1000
Hydrogen Peroxide (35% active)	26.000

ii. Part B

<u>Description</u>	<u>Wt %</u>
Emulsion Base	44.5000
Demineralised water	40.0000
Sodium sulphite	0.2000
Ascorbic acid	0.2000
Ammonia (30% active)	11.000
Ammonium acetate	3.0000
EDTA chelating agent	4.0000

Part A and Part B are mixed in a 1:1 ratio immediately before application to the hair.

e. Product 5i. Part A

<u>Description</u>	<u>Wt %</u>
Emulsion Base	35.0000
Demineralised water	38.6500
DTPA chelating agent	0.2500
HEDP chelating agent	0.1000
Hydrogen Peroxide (35% active)	26.000

ii. Part B

<u>Description</u>	<u>Wt %</u>
Emulsion Base	44.5000
Demineralised water	40.0000
Sodium sulphite	0.2000
Ascorbic acid	0.2000
Ammonia (30% active)	11.000
Ammonium acetate	3.0000
EDDS chelating agent	3.8000

Part A and Part B are mixed in a 1:1 ratio immediately before application to the hair

APPENDIX 2
MEASUREMENT OF LIGHTENING AND COLOUR UPTAKE

The instrument used to measure the colour and lightening of the hair was the Hunter LabScan XE spectrophotometer. The settings used were the area view = 12.7mm and the port size = 17.0mm. Before each use the instrument was calibrated using a black tile and a white tile. A check for the drift of colour over time was performed each week using a calibrated green tile provide by Hunter.

To measure the colour the hair switch to be measured was placed in an appropriately sized switch holder and made sure that the springs pulled the hair taut and flat against the holder. The hair should be mounted on the white side of the switch holder, to ensure measurement against a white background. The sample was placed face down over the measuring port and the measurement was made. A total of eight readings are taken for all tests; 4 on one side of the switch, and four on the other side, moving the hair switch along its length between readings.

The data generated gives the Lightening level (*L*), and the change in colour (*a* and *b* values). Relevant to this work is the *L* value, the amount of lightening from the oxidant.

APPENDIX 3
MEASUREMENT OF FIBRE DAMAGE USING FT-IR

Damage caused to the hair was assessed by the Fourier Transform Infrared (FT-IR) method, which has been established to be suitable for studying the effects of oxidative treatments on hair (Strassburger, J., *J. Soc. Cosmet. Chem.*, 36, 61-74 (1985); Joy, M. & Lewis, D.M., *Int. J. Cosmet. Sci.*, 13, 249-261 (1991); Signori, V. & Lewis, D.M., *Int. J. Cosmet. Sci.*, 19, 1-13 (1997)).

In particular, the above authors have shown that the method is suitable for quantifying the amount of cysteic acid that is produced from the oxidation of cystine. In general, the oxidation of cystine is thought to be a suitable marker by which to monitor the overall oxidation of the keratinous part of the fiber.

Thus, the measurement of cysteic acid units by FT-IR is often used to study the effects of oxidative treatments or environmental oxidation upon keratin protein containing fibers such as hair and wool.

Signori & Lewis (1997) have shown that FT-IR using a diamond Attenuated Total Internal Reflection (ATR) cell is a sensitive and reproducible way of measuring the cysteic acid content of single fibers and bundles. They have shown that this technique is more suitable than using the FT-IR method in simple transmission or microscope modes. They have also shown that the diamond cell ATR was significantly more sensitive and reproducible than the ZnSE cell.

Hence, the method that we have employed to measure the cysteic acid content of multiple fiber bundles and full hair switches, is based upon the FT-IR diamond cell ATR method employed by Signori and Lewis (1997).

The detailed description of the method used for assessing hair damage follows:

A Perkin Elmer Spectrum 1[®] Fourier Transform Infrared (FT-IR) system equipped with a diamond Attenuated Total Internal Reflection (ATR) cell was used to measure the cysteic acid concentration in human hair. In our particular method, hair switches of various sizes and colours were used. The switches were platted (~1 plait per cm) in order to minimize variations in surface area of contact between readings. Six readings per switch

were taken (~1/3 and 2/3s down the switch on both sides), and an average calculated. Backgrounds were collected every 4 readings, and an ATR cell pressure of 1N/m was employed. The cell was cleaned with ethanol between each reading, and a contamination check performed using the monitor ratio mode of the instrument. As prescribed by Signori & Lewis (1997), a normalized double derivative analysis routine was used. The original spectra were initially converted to absorbance, before being normalized to the 1450cm⁻¹ band (the characteristic and invariant protein CH₂ stretch). This normalized absorbance was then twice derivatised using a 13 point averaging. The value of the 1450cm⁻¹ normalized 2nd derivative of the absorbance at 1040cm⁻¹ was taken as the relative concentration of cysteic acid. This figure was multiplied by -1x10⁻⁴ to recast it into suitable units. It was found that virgin human hair produced a value of ~20 cysteic acid units, and heavily oxidized hair produced values of >170. The following instrumental conditions were employed:

Spectral Resolution	4cm ⁻¹
Data Interval	0.7cm ⁻¹
Mirror Scan Speed	0.2cms ⁻¹
Number of Background Scans	20
Number of Sample Scans	20
Scan Range	4000cm ⁻¹ to 600cm ⁻¹

Using these instrumental conditions and the 2nd derivative analysis routine, it was found that the sensitivity and reproducibility of the method in the range 10 to 150 cysteic acid units, are both ~±5-10%.